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(54) Title: PREVENTION OF RETINAL INJURY AND DEGENERATION BY SPECIFIC FACTORS

(57) Abstract

Photoreceptor injury or cell death (retinal degeneration) is prevented by the introduction into the living mammalian eye of specific, survival-promoting factors. These specific factors prevent damage and degeneration of photoreceptors when introduced into the living eye prior to, during or after exposure to the damaging effects of light and delay photoreceptor damage caused by inherited disease.

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## PREVENTION OF RETINAL INJURY AND DEGENERATION BY SPECIFIC FACTORS

5 This application is a continuation-in-part of United States Patent Application Serial No. 07/836,090 filed February 14, 1992 which is a continuation-in-part of Serial No. 07/691,612 filed April 25, 1991, which is a continuation-in-part of Serial No. 07/570,657 filed August 20, 1990 which is a continuation-in-part of Serial No. 07/400,591 filed on August 30, 1989.

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### INTRODUCTION

15 The present invention relates to a method of preventing or delaying retinal degeneration caused by exposure to light or other environmental trauma, or by any pathological condition wherein death or injury of retinal neurons or photoreceptors occurs. It is based on the discovery that specific survival promoting factors, when introduced into the living mammalian eye, prevent damage and degeneration of photoreceptors caused by light and on 20 the further discovery that such factors can delay photoreceptor degeneration associated with inherited diseases of the retina.

### BACKGROUND OF THE INVENTION

25 Trophic factors play a major role in neuronal survival and growth during development, in addition to the maintenance of differentiated neurons. Such factors also appear to play a role in the survival and

regeneration of injured neurons in the central as well as in the peripheral nervous system.

In mammals, a number of diseases of the retina involve injury or degeneration of retina-associated neurons. Trophic factors capable of 5 rescuing these neurons may provide useful therapies for the treatment of such diseases.

There is some evidence that the neurotrophic factor NGF (nerve growth factor) enables axonal regrowth of retinal ganglion cells in response to optic nerve section. (Carmignoto, et al. J. Neuroscience 9 (1989): 1263-1272).

10 Extracts from pig brain stimulate neurite outgrowth in retinal explants; the outgrowth was shown to be due to a factor other than NGF. (Turner, et al. Dev. Brain Res. 6 (1983) 77-83). BDNF (brain derived neurotrophic factor) purified from brain promotes the survival of retinal ganglion cells in vitro. (Johnson, et al. J. Neuroscience 6 (1986): 3031-3038; Thanos, et al. Eur. J. 15 Neuroscience 1(1989): 19-26.) Other workers have reported that retinal ganglion cells could be maintained by extracts from the neonatal superior colliculus and that a factor purified from such extracts promotes the survival and growth of retinal ganglion cells in vivo. (Schultz, et al. J. Neurochemistry 55(1990): 832-303). Moreover, fibroblast growth factors 20 promote the survival of adult rat ganglion cells after application to transected optic nerves (Sievers, et al., Neurosci. Let. 76 (1987):157-162).

In addition to the survival of retinal ganglion cells, there is some evidence that certain cellular factors may promote the survival and/or 25 regeneration of photoreceptors. Photoreceptors consist of rods and cones which are the photosensitive cells of the retina. The rods contain rhodopsin, the rod photopigment, and the cones contain 3 distinct photopigments, which

respond to light and ultimately trigger a neural discharge in the output cells of the retina, the ganglion cells. Ultimately, this signal is registered as a visual stimulus in the visual cortex.

The retinal pigment epithelial (RPE) cells produce, store and transport 5 a variety of factors that are responsible for the normal function and survival of photoreceptors. RPE are multifunctional cells that transport metabolites to the photoreceptors from their blood supply, the chorio capillaris of the eye. The RPE cells also function to recycle vitamin A as it moves between the photoreceptors and the RPE during light and dark adaptation. RPE cells also 10 function as macrophages, phagocytizing the rhythmically-shed tips of the outer segments of rods and cones. Various ions, proteins and water move between the RPE cells and the interphotoreceptor space, and these molecules ultimately effect the metabolism and viability of the photoreceptors.

RCS (Royal College of Surgeons) rats, which have an inherited retinal 15 dystrophy due to mutant gene expression in the RPE, with secondary photoreceptor cell death (Mullen & LaVail, Science 192 (1976):799-801), provide a useful model system to study the role of trophic factors on the retina. Using such rats, delay of photoreceptor degeneration caused by the inherited defect was obtained by the juxtaposition of normal RPE cells to the 20 photoreceptors before their degeneration both in experimental chimeras (Mullen & LaVail, Science 192 (1976):799-801) and in transplantation experiments (Li & Turner, Exp. Eye Res. 47: 911-917, 1988). In these experiments, the "rescue" extended beyond the boundaries of the normal RPE cells. These findings suggested the presence of a diffusible factor produced 25 by the RPE cells. It was subsequently determined that subretinal or intravitreal injection of basic fibroblast growth factor (bFGF) resulted in

extensive photoreceptor rescue in RCS rats (Faktorovich, et al., *Nature* 347 (1990):83-86). Basic FGF was also shown to induce retinal regeneration from the RPE in chick embryos (Park & Hollenberg, *Dev. Biol.* 134 (1989): 201-205).

Although the results obtained with injection of bFGF were 5 encouraging, therapeutic applications of bFGF could be very limited. Given its mitogenic and angiogenic properties, harmful side effects can be expected. As an example, intravitreal injections of bFGF consistently result in numerous invading macrophages in the inner retina, and occasionally produce a massive proliferative vitreoretinopathy (Faktorovich, et. al, *Nature* 347 (1990):83-86). Finally, bFGF is unable to remedy one particular defect seen in 10 RCS rats, which is the inability of the RPE to phagocytosize degenerated neurons.

More limited rescue of photoreceptors in RCS rats has been reported with the injection of phosphate buffered saline (PBS) (Silverman & Hughes, 15 *Current Eye Res.* 9 (1990): 183-191; Faktorovich, et. al, *Nature* 347 (1990):83-86), as well as in surgical controls. Such studies indicated a localized effect caused by the possible release of protective factors from RPE or other cells damaged during injection. In such instances, however, the level of rescue differed quantitatively from that obtained using bFGF, i.e. it was much more 20 restricted to the area of the needle track.

In the albino rat, normal illumination levels of light, if continuous, can cause complete degeneration of photoreceptors. Results obtained using such rats as a model to identify survival enhancing factors appear to correlate well with data obtained using RCS rats. Moreover, different factors can be 25 compared and complications can be assessed more quickly in the light damage model than can be assessed by testing factors in models which are

based on the slowly evolving dystrophy of the RCS rat. Furthermore, since the mechanism of cell death in light damage is better defined than that in the RCS rats, the results in the light damage model can be more readily applied to human diseases.

5       Using albino rats, it has been determined that a number of agents, when administered systemically (intraperitoneally) can be used to ameliorate retinal cell death or injury caused by exposure to light. In general, exposure to light generates oxygen free radicals and lipid peroxidation products. Accordingly, compounds that act as antioxidants or 10      as scavengers of oxygen free radicals reduce photoreceptor degeneration. 10      Agents such as ascorbate (Organisciak et al, Investigative Ophthalmology & Visual Science 26 (1985):1580-1588), flunarizine (Edward, et al., Laboratory Science 109(1991):554-562) and dimethylthiourea (Lam, et al., Archives of Ophthalmology 108 (1990):1751-1757) have been used to ameliorate the 15      damaging effects of constant light. There is no evidence, however, that these compounds will act to ameliorate other forms of photoreceptor degeneration and their administration can generate potentially harmful side effects. 15      Further, these studies are limited because they utilize systemic delivery. Such delivery often provides an inadequate means of assessing the efficacy of 20      a particular factor. It is difficult to assess the amount of agent that actually reaches the retina. A large amount of agent must be injected to attain a sufficient concentration at the site of the retina. In addition, systemic toxic effects may result from the injection of certain agents.

25      Other than the use of bFGF to delay inherited photoreceptor degeneration in RCS rats, there is no demonstrated use of any specific neurotrophic or other cellular factor to prevent injury or death of mammalian

photoreceptors. In copending U. S. application 07/400,591 which is incorporated by reference herein, a BDNF expressing clone was isolated from a retinal cDNA library. Based on that discovery, as well as the expression for the first time of purified BDNF using recombinant technology, a means was 5 provided for the use of a purified neurotrophic factor for the treatment of diseases such as retinitis pigmentosa and other retinal degenerations. As described in greater detail below, the efficacy of BDNF, in addition to other neurotrophic and cellular factors, has been demonstrated, providing the first pharmacological means to treat most forms of inherited, age-related or 10 environmentally-induced retinal degenerations.

#### SUMMARY OF THE INVENTION

An object of the present invention is to provide a method of preventing 15 injury or death of retinal neurons.

Another object of the invention is to provide a method of treating pathological diseases wherein degeneration of the retina occurs.

Yet another object of the invention is to provide a method of treating the living eye prior to or following exposure to light or other environmental 20 trauma thereby preventing degeneration of retinal cells.

A further object of the present invention is to provide a method of preventing photoreceptor injury and degeneration in the living eye.

Another object of the invention is to provide a method of protecting 25 retinal neurons without the induction of side effects.

Another object of the invention is to provide a method of allowing 25 injured photoreceptors to recover or regenerate.

Another object of the invention is to provide an *in vivo* assay system for assessing the survival-promoting activity of neurotrophic and other cellular factors on retinal neurons and photoreceptors.

These and other objects are achieved by treating the eye with an effective amount of a neurotrophic factor such as brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), neurotrophin-3 (NT-3) or neurotrophin-4 (NT-4), or a cellular factor such as acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF) plus heparin, aFGF plus heparin, interleukin-1 beta (IL-1 $\beta$ ), tumor necrosis factor-alpha (TNF- $\alpha$ ) and insulin-like growth factor-2 (IGF-2). Similar effects, but to a lesser degree, may be achieved using other neurotrophic or cellular factors that may, alone, or in combination with other factors described herein, have therapeutically beneficial effects. Such factors include nerve growth factor (NGF), heparin, epidermal growth factor (EGF), platelet derived growth factor (PDGF) and insulin-like growth factor-1 (IGF-1).

#### DESCRIPTION OF THE FIGURES

FIG. 1 is a histogram illustrating the degree of photoreceptor rescue obtained using the neurotrophic and cellular factors.

FIG. 2 is a histogram illustrating the ONL thickness obtained using the neurotrophic and cellular factors.

FIG. 3 is a composite of three light micrographs showing a) control retina from a rat not exposed to light; b) control retina from a rat exposed to light after PBS injection; and c) BDNF-treated rat retina after exposure to light.

FIG. 4 is a histogram illustrating the degree of macrophage incidence observed using the neurotrophic and cellular factors.

#### DETAILED DESCRIPTION OF THE INVENTION

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The present invention provides for the utilization of neurotrophic, as well as other cellular factors to delay, prevent or rescue photoreceptors, as well as other retinal cells, including neurons or supportive cells (e.g. Müller cells or RPE cells) from injury and degeneration. Other retinal neurons include, but are not limited to, retinal ganglion cells, displaced retinal ganglion cells, amacrine cells, displaced amacrine cells, horizontal and bipolar neurons.

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As contemplated herein, neurotrophic or other cellular factors are utilized to treat any condition which results in injury or death of photoreceptors or other retinal cells. Examples of conditions include: retinal detachment; age-related and other maculopathies, photic retinopathies; surgery-induced retinopathies (either mechanically or light-induced); toxic retinopathies including those resulting from foreign bodies in the eye; diabetic retinopathies; retinopathy of prematurity; viral retinopathies such as CMV or HTV retinopathy related to AIDS; uveitis; ischemic retinopathies due to venous or arterial occlusion or other vascular disorders; retinopathies due to trauma or penetrating lesions of the eye; peripheral vitreoretinopathy; and inherited retinal degenerations.

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The factors which are useful in practicing this invention include one or more neurotrophic factor such as brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), neurotrophin-3 (NT-3), neurotrophin-4

(NT-4) or functional derivatives or analogs thereof, or one or more cellular factor such as basic fibroblast growth factor (bFGF) plus heparin, acidic fibroblast growth factor (aFGF), aFGF plus heparin, leukemia inhibitory factor (LIF), interleukin-1 beta (IL-1 $\beta$ ), tumor necrosis factor-alpha (TNF- $\alpha$ ), and insulin-like growth factor-2 (IGF-2), or functional derivatives or analogs thereof. Other factors that appears to be effective, but to a lesser extent, include nerve growth factor (NGF), heparin, epidermal growth factor (EGF), platelet derived growth factor (PDGF) and insulin-like growth factor-1 (IGF-1). A functional derivative of a factor is a compound which is an analog or an active fragment of the compound or its analog. Combinations of the neurotrophic factors and cellular factors may also be used to achieve optimum results.

Each of the factors utilized may be obtained by methods known by those skilled in the art. For example, they may be purified from a natural source. Alternatively, they may be made by recombinant means utilizing available sequence data. (See, for example, for CNTF; Masiakowski, et al. J. Neurochemistry 57(1991): 1003-1012; NT-3; Maisonpierre, et al. Science 247(1990): 1446-1451). A recombinant bacteriophage (HG7-2), containing a human genomic sequence related to neurotrophin-4 was deposited on 20 August 22, 1991 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, and assigned accession number ATCC 75070.

Of particular suitability in practicing the subject invention are the neurotrophic factors. As used herein, neurotrophic factors are proteins responsible for the development and maintenance of the nervous system. 25 Widespread neuronal cell death accompanies normal development of the

central and peripheral nervous systems, and apparently plays a crucial role in regulating the number of neurons which project to a given target field (Berg, D. K., 1982, *Neuronal Development* 297-331). Ablation and transplantation studies have shown that neuronal cell death results from the competition among neurons for limiting amounts of survival factors ("neurotrophic factors"). The important neurotrophic factors identified to date are NGF, BDNF, CNTF, NT-3 and NT-4.

In a preferred embodiment of the invention, BDNF is utilized to treat any condition which results in injury or death of photoreceptors or other retina-related cells. With the molecular cloning of BDNF, as well as the resultant production and purification of purified recombinant BDNF, as described in USSN 400,591, it became possible to determine the physiological effects of BDNF on developing neurons, as well as to quantify the levels of BDNF in tissues by immunoassay and to localize BDNF in tissues using immunocytochemistry. Furthermore, a BDNF cDNA was found in a retinal library and BDNF mRNA was found to be expressed in adult retinas (Maisonpierre, et al. *Neuron*, 5(1990): 501-509); suggesting production of the protein in the retina and a possible role for the factor in promoting retinal cell survival.

As described herein, treatment of the eye with BDNF results in the increased survival of photoreceptors upon exposure to environmental trauma such as light. Surprisingly, BDNF does not cause the influx of macrophages observed when treating the retina with bFGF. Furthermore, BDNF is not anticipated to have the side effects of bFGF as it does not have similar angiogenic or mitogenic properties.

In another preferred embodiment, ciliary neurotrophic factor (CNTF) is used to prevent or delay photoreceptor degeneration. CNTF, like BDNF, effectively protects photoreceptors without macrophage influx and the mitogenic and angiogenic properties of bFGF.

5 In another preferred embodiment, NT-4 is used to prevent photoreceptor degeneration. As with BDNF and CNTF, NT-4 effectively protects photoreceptors without macrophage influx or any mitogenic or angiogenic effects.

10 In another embodiment, leukemia inhibitory factor (LIF) is used to prevent photoreceptors from injury.

In still another embodiment, aFGF is used to prevent photoreceptor degeneration. This factor, unlike bFGF, appears to provide protection without the influx of macrophages observed when bFGF is used.

15 In yet another embodiment, bFGF is used in conjunction with a compound that suppresses the influx of macrophages observed using bFGF alone. Heparin appears to be useful for this purpose. Combinations of heparin and bFGF prevent photoreceptor injury without macrophage influx, and heparin enhances the action of aFGF, as well as bFGF (see Figure 4).

20 In another embodiment, other factors such as IL-1 $\beta$  and TNF- $\alpha$  provide a substantial amount of retinal protection. IL-1 $\beta$  however, has been observed to cause folding and rosette formation and a somewhat greater incidence of macrophages than is observed in control retinas or those protected with BDNF or CNTF. Use of TNF- $\alpha$  may also be associated with a slightly greater than normal incidence of macrophages.

25 In additional embodiments, the light damage model may be used to evaluate the effect of various survival-promoting factors on the retina. As

shown herein, the intravitreal administration of various factors into the eyes of albino rats enabled the rapid assessment of both the ability of the factors to rescue photoreceptors from degeneration and the side effects, such as incidence of macrophages, associated with each factor. Although the model 5 described herein is the albino rat, the eyes of other albino mammals, such as mice and rabbits, are also useful for this purpose.

Although the light damage model has been used previously to assess the effect of various agents such as antioxidants on the retina, such studies have always been conducted using systemic (intraperitoneal) administration. 10 As described herein, the intravitreal injection of potential survival promoting factors represents a novel method of assessing factors, with several advantages over systemic application. The amount of any specific agent that reaches the retina can be more accurately determined, since the eye is a round, relatively contained structure and the agent is injected directly into it. Moreover, the 15 amount of agent that need to be injected is minuscule compared to systemic injections. For example, a single microliter in volume (about 1 microgram of agent) is used for intravitreal injection, as compared to one to several milliliters (ten to several hundred milligrams of agent) necessary for systemic injections. In addition, the intravitreal route of administration avoids the 20 potentially toxic effect of some agents.

According to the present invention, the factors used herein prevent the degeneration of retinal cells. It has been further observed that when animals that have been exposed to damaging light are returned to normal light, they will regenerate their inner and outer segments. Thus, the factors of the 25 present invention are able not only to protect and prevent photoreceptors from degeneration, but also to promote regeneration of retinal cells.

The factors of the present invention can be delivered to the eye through a variety of routes. They may be delivered intraocularly, by topical application to the eye or by intraocular injection into, for example the vitreous or subretinal (interphotoreceptor) space. Alternatively, they may be delivered locally by insertion or injection into the tissue surrounding the eye. They may be delivered systemically through an oral route or by subcutaneous, intravenous or intramuscular injection. Alternatively, they may be delivered by means of a catheter or by means of an implant, wherein such an implant is made of a porous, non-porous or gelatinous material, including membranes such as silastic membranes or fibers, biodegradable polymers, or proteinaceous material. The factors may be administered prior to the onset of the condition, to prevent its occurrence, for example, during surgery on the eye, or immediately after the onset of the pathological condition or during the occurrence of an acute or protracted condition.

15 The factors of the present invention may be modified to enhance their ability to penetrate the blood-retinal barrier. Such modifications may include increasing their lipophilicity by, for example, glycosylation, or increasing their net charge by methods known in the art.

20 The factors may be delivered alone or in combination, and may be delivered along with a pharmaceutically acceptable vehicle. Ideally, such a vehicle would enhance the stability and/or delivery properties. The invention also provides for pharmaceutical compositions containing the active factor or fragment or derivative thereof, which can be administered using a suitable vehicle such as liposomes, microparticles or microcapsules.

25 In various embodiments of the invention, it may be useful to use such compositions to achieve sustained release of the active component.

The amount of factor which will be effective in the treatment of a particular disorder or condition will depend of the nature of the disorder or condition and can be determined by standard clinical techniques.

EXAMPLE 1.

5

Use of Neurotrophic and Cellular Factors To Prevent Light  
Induced Photoreceptor Injury

Albino rats of either the F344 or Sprague-Dawley strain were used at 10 2-5 months of age. The rats were maintained in a cyclic light environment (12 hr on: 12 hr off at an in-cage illuminance of less than 25 ft-c) for 9 or more days before being exposed to constant light. The rats were exposed to 1 or 2 weeks of constant light at an illuminance level of 115-200 ft-c (most rats received 125-170 ft-c) provided by two 40 watt General Electric "cool-white" 15 fluorescent bulbs with a white reflector that was suspended 60cm above the floor of the cage. During light exposure, rats were maintained in transparent polycarbonate cages with stainless steel wire-bar covers.

Two days before constant light exposure, rats anesthetized with a 20 ketamine-xylazine mixture were injected intravitreally with 1  $\mu$ l of the various factors dissolved in phosphate buffered saline (PBS) at a concentration of 50-1000 ng/ $\mu$ l. The injections were made with the insertion of a 32 gauge needle through the sclera, choroid and retina approximately midway between the ora serrata and equator of the eye. The factor-injected animals were compared to either uninjected littermates or to those that received intravitreal 25 injections of 1  $\mu$ l of PBS alone, as well as to animals that were not exposed to constant light. Controls included the injection of 1  $\mu$ l of PBS alone, or the

insertion of a dry needle with no injection. In all cases, the injections were made into the superior hemisphere of the eye.

Immediately following constant light exposure, the rats were killed by overdose of carbon dioxide followed immediately by vascular perfusion of 5 mixed aldehydes. The eyes were embedded in epoxy resin for sectioning at 1  $\mu\text{m}$  thickness to provide sections of the entire retina along the vertical meridian of the eye. The degree of light-induced retinal degeneration was quantified by two methods. The first was by measuring outer nuclear layer (ONL) thickness, which is used as an index of photoreceptor cell loss. A 10 mean ONL thickness was obtained from a single section of each animal with the aid of a Bioquant morphometry system. In each of the superior and inferior hemispheres, ONL thickness was measured in 9 sets of 3 measurements each (total of 27 measurements in each hemisphere). Each set was centered on adjacent 440- $\mu\text{m}$  lengths of retina (the diameter of the 15 microscope field at 400X magnification). The first set of measurements was taken at approximately 440 $\mu\text{m}$  from the optic nerve head, and subsequent sets were located more peripherally. Within each 440- $\mu\text{m}$  length of retina, the 3 measurements were made at defined points separated from one another by 75  $\mu\text{m}$  using an eyepiece micrometer. In this way, the 54 measurements in the 20 two hemispheres sampled representative regions of almost the entire retinal section. The results obtained with each of the factors tested are summarized in Figure 1.

The second method of assessing the degree of photoreceptor rescue was by a 0-4+ pathologist's scale of rescue, 4+ being maximal rescue and 25 almost normal retinal integrity. The degree of photoreceptor rescue in each section, as based on comparison to the control eye in the same rat, was scored

by four individuals. This method has the advantage of considering not only the ONL thickness, but also more subtle degenerative changes to the photoreceptor inner and outer segments, as well as spatial degenerative gradients within the eye. Data obtained from this method is summarized in 5 Figure 2. The number of eyes examined for each factor was 10 or more, except for insulin and laminin, which was 6 each.

## RESULTS AND DISCUSSION

10 The data obtained using the light damage model of photoreceptor injury is presented in Figures 1, 2 and 3. Neurotrophic factors BDNF, NT-4 and CNTF provided a high degree of rescue. The factors LIF, bFGF, aFGF, bFGF plus heparin, aFGF plus heparin, TNF- $\alpha$ , IL-1 $\beta$ , NT-3 and IGF-2 also provided a significant amount of rescue. Notably, all of the factors other 15 than bFGF enhanced survival without inducing a high incidence of macrophages, as seen in Figure 4 (IL-1 $\beta$  and TNF- $\alpha$  were associated with a slightly higher incidence of macrophages). Some factors actually suppressed the incidence of macrophages as compared to control retinas (retinas in the same animal that were injected with PBS). Such factors 20 included BDNF, aFGF, and bFGF plus heparin.

Acidic fibroblast growth factor (aFGF), which had previously been reported to be ineffective as compared to bFGF in the RCS rat, was shown to provide significant protection of photoreceptors in the light-damage model. In addition, the influx of macrophages normally observed with injections of

bFGF were not seen when bFGF was used in combination with heparin, thus eliminating a side effect that potentially would have obviated the use of bFGF.

Some degree of rescue, although to a lesser extent, was observed with  
5 heparin, PDGF, NGF, EGF and IGF-1. The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to  
10 fall within the scope of the appended claims.

We claim:

1. A method of reducing or preventing degeneration of retinal neurons in a mammal caused by exposure to light or other environmental trauma comprising administering to the mammal, prior to, during or following such exposure, a therapeutically effective dose of neurotrophic factor.
2. The method of claim 1 wherein said neurotrophic factor is brain derived neurotrophic factor, ciliary neurotrophic factor, neurotrophin-3, neurotrophin-4, or a combination thereof.
3. The method of claim 2 wherein said retinal neurons are photoreceptors.
4. The method of claim 3 wherein said administration is intraocular.
5. The method of claim 4 wherein said administration is into the vitreous or into the subretinal (interphotoreceptor) space.
6. The method of claim 3 wherein said administration is systemic delivery.
7. The method of claim 6 wherein said neurotrophic factor has been modified in such a way as to increase its ability to be transported across the blood-retinal barrier.
8. The method of claim 7 wherein said modification comprises increasing the lipophilicity of the factor.

9. The method of claim 7 wherein said modification comprises glycosylation of the factor.
10. The method of claim 7 wherein said modification comprises increasing the net positive charge on said factor.
11. The method of claim 6 wherein said systemic delivery is by an oral route.
12. The method of claim 7 wherein said systemic delivery is by subcutaneous, intravenous or intramuscular injection.
13. A method of preventing or reducing degeneration of retinal neurons in a mammal caused by exposure to light or other environmental trauma comprising administering to the mammal, prior to, during or following said exposure, a therapeutically effective dose of one or more factors selected from the group consisting of leukemia inhibitory factor (LIF), acidic fibroblast growth factor (aFGF), bFGF plus heparin, aFGF plus heparin, interleukin-1 beta (IL-1 $\beta$ ) and tumor necrosis factor-alpha (TNF- $\alpha$ ).
14. The method of claim 13 wherein said retinal neurons are photoreceptors.
15. The method of claim 14 wherein said administration is intraocular.
16. The method of claim 15 wherein said administration is into the vitreous or into the subretinal (interphotoreceptor) space.

17. The method of claim 14 wherein said administration is delivered systemically.
18. The method of claim 17 wherein said systemic delivery is by an oral route.
19. The method of claim 18 wherein said systemic delivery is by subcutaneous, intravenous or intramuscular injection.
20. A method of reducing or preventing degeneration of retinal neurons in a mammal having a pathological condition wherein retinal degeneration occurs, comprising administering to said mammal a therapeutically effective dose of a neurotrophic factor.
21. The method of claim 20 wherein said pathological condition is retinal detachment, age-related or other maculopathies, photic retinopathies, surgery-induced retinopathies (either mechanically or light-induced), toxic retinopathies, diabetic retinopathies, retinopathy of prematurity, viral retinopathies such as CMV or HIV retinopathy related to AIDS; uveitis; ischemic retinopathies due to venous or arterial occlusion or other vascular disorder, retinopathies due to trauma or penetrating lesions of the eye, peripheral vitreoretinopathy or inherited retinal degenerations.

22. The method of claim 21 wherein said neurotrophic factor is brain-derived neurotrophic factor, ciliary neurotrophic factor, neurotrophin-3, neurotrophin-4 or a combination thereof.
23. The method of claim 22 wherein said retinal neurons are photoreceptors.
24. The method of claim 23 wherein said administration is intraocular.
25. The method of claim 24 wherein said administration is into the vitreous or into the subretinal (interphotoreceptor) space.
26. The method of claim 23 wherein said administration is by systemic delivery.
27. The method of claim 26 wherein said systemic delivery is by an oral route.
28. The method of claim 27 wherein said systemic delivery is by subcutaneous, intravenous or intramuscular injection.
29. A method of reducing or preventing degeneration of retinal neurons in a mammal having a pathological condition wherein retinal degeneration occurs, comprising administering to said mammal a therapeutically effective dose of one or more factors selected from the group consisting of leukemia inhibitory factor (LIF), acidic fibroblast growth factor (aFGF), bFGF plus heparin, aFGF plus heparin, IL-1 $\beta$ , TNF- $\alpha$  and IGF-2.

30. The method of claim 29 wherein said retinal neurons are photoreceptors.
31. The method of claim 30 wherein said administration is intraocular.
32. The method of claim 31 wherein said administration is into the vitreous or into the subretinal (interphotoreceptor) space.
33. The method of claim 30 wherein said administration is systemic delivery.
34. The method of claim 33 wherein said systemic delivery is by an oral route.
35. The method of claim 34 wherein said systemic delivery is by subcutaneous, intravenous or intramuscular injection.
36. A method of assessing the survival-promoting ability of an agent on retinal neurons or photoreceptors comprising
  - (i) injecting the agent intravitreally into an albino mammal eye, prior to, during, or after exposure of the mammal to continuous light,
  - (ii) evaluating the injected eye for degeneration of retinal neurons or photoreceptors as compared to a control eye exposed to the same light in the absence of injection of the agent;wherein decreased retinal degeneration as compared to the control eye correlates positively with survival-promoting ability of the agent.

37. The method of claim 36 wherein said mammal is a rat.
38. The method of claim 36 wherein said control eye is in the same mammal as the intravitreally injected eye.

FIG. 1

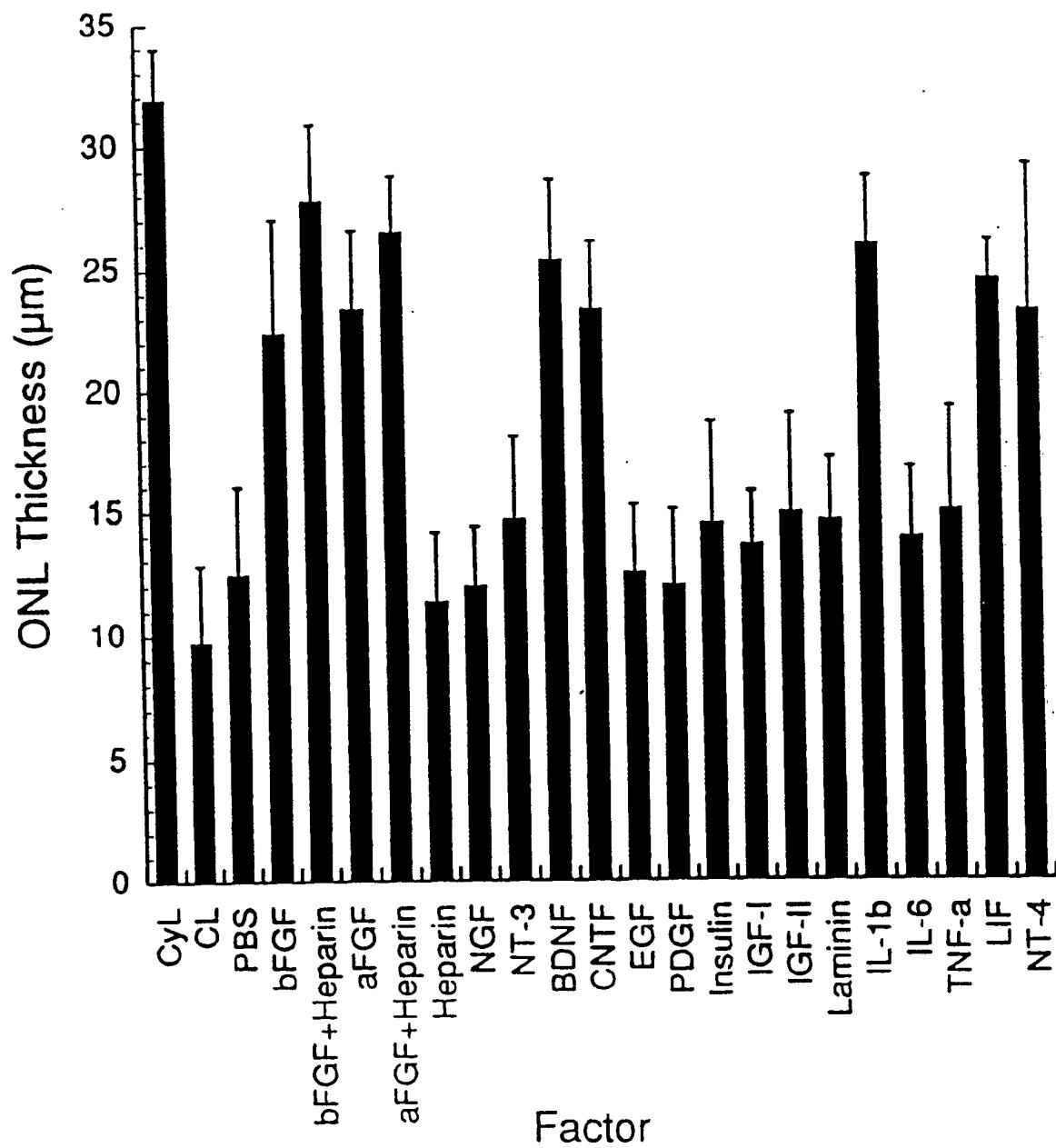
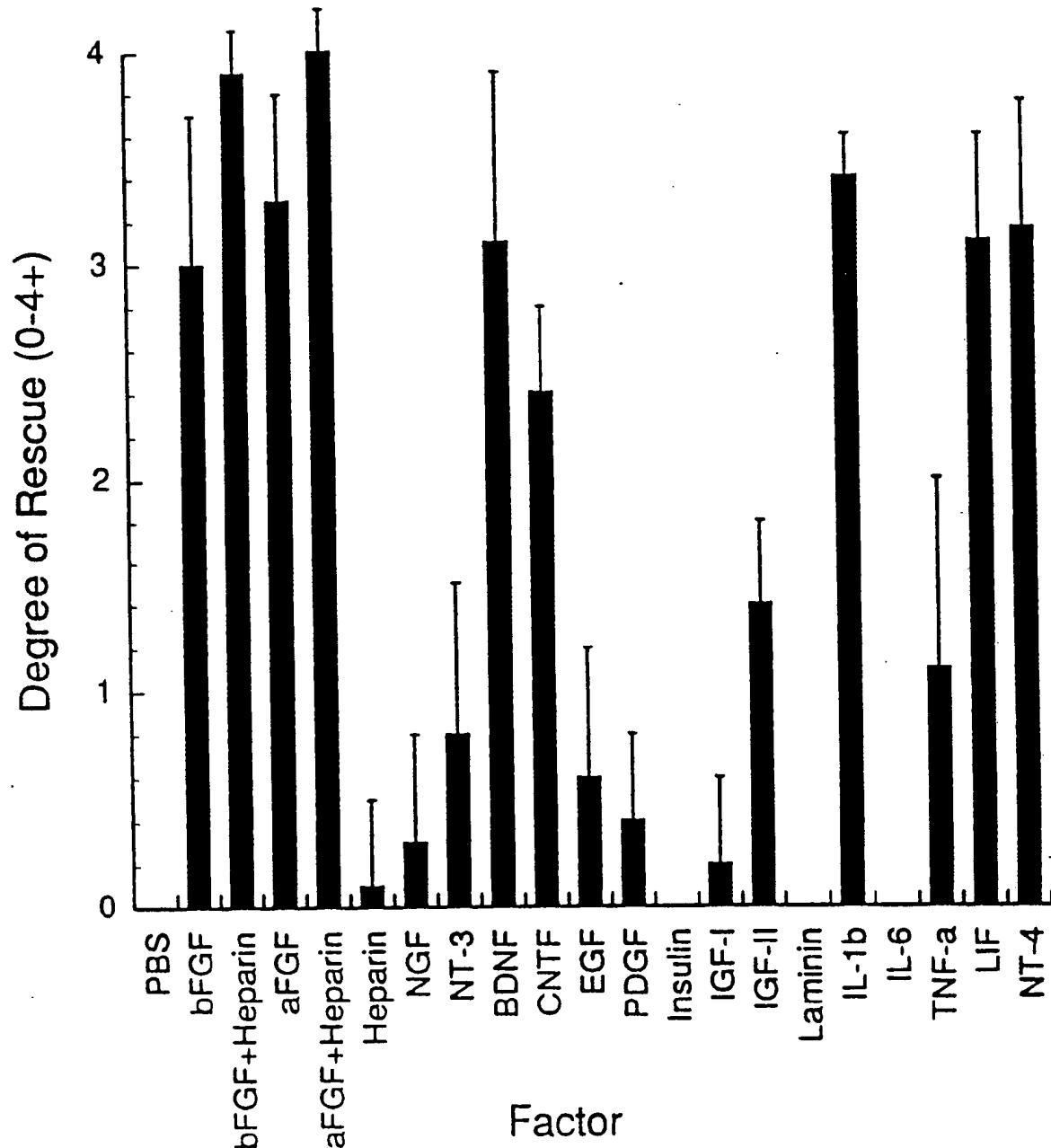
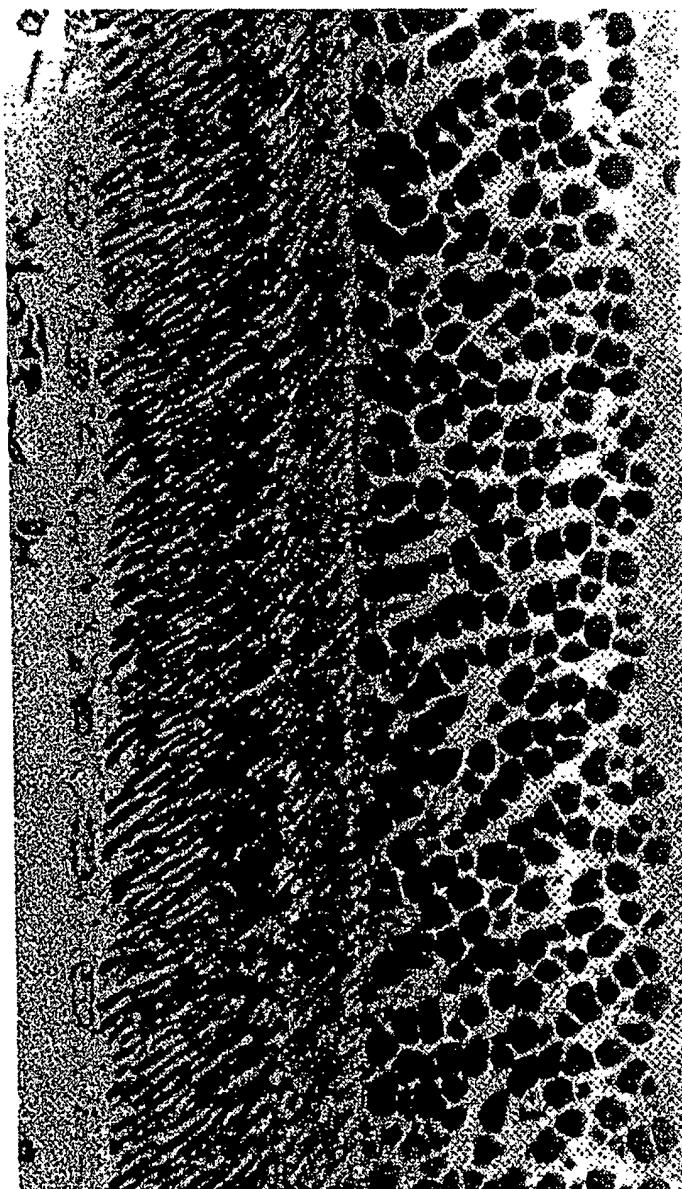


FIG. 2



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FIG. 3A



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FIG. 3B

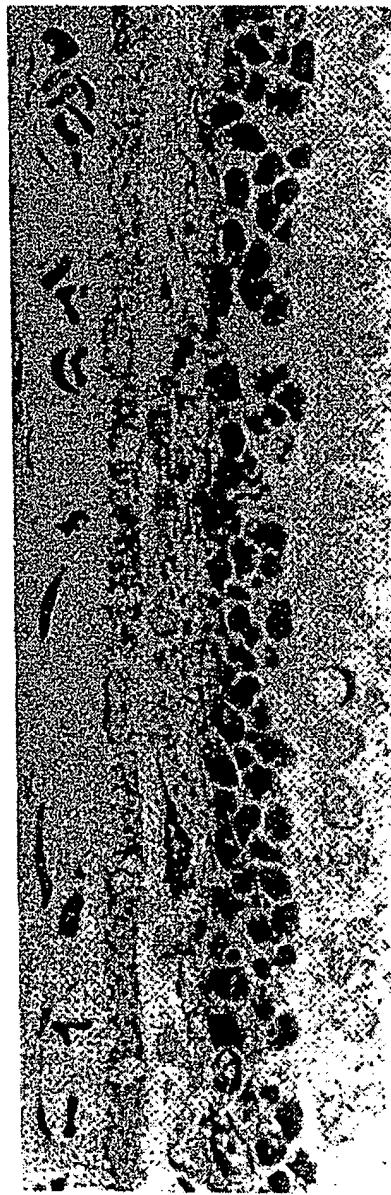


FIG. 3C

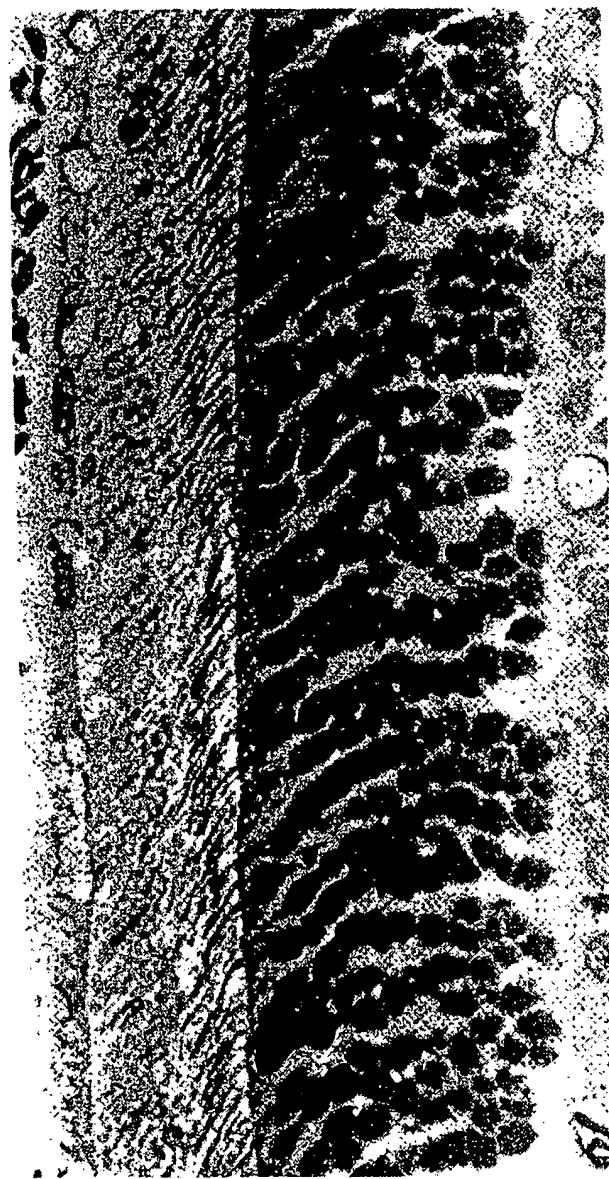
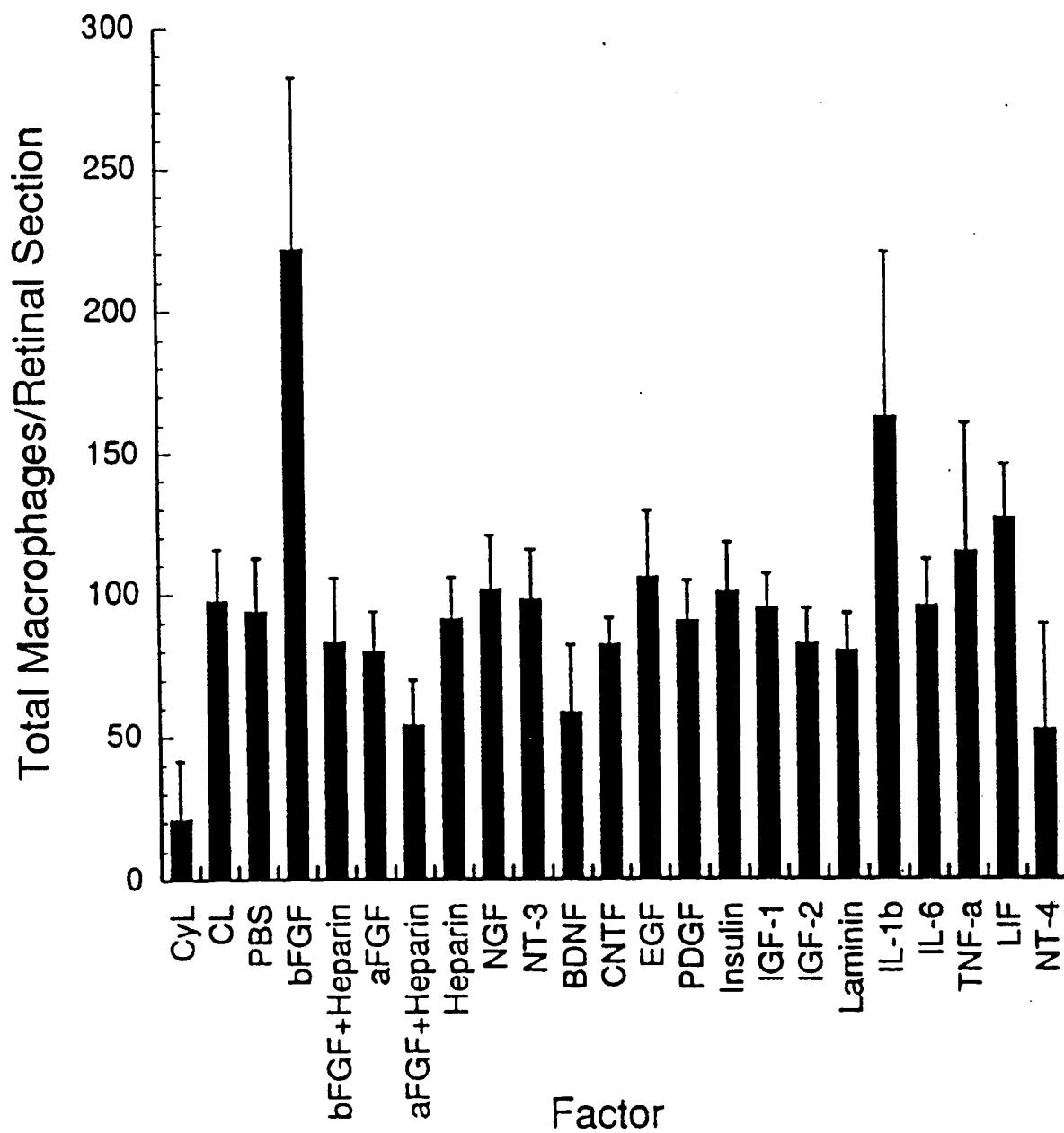


FIG. 4



## A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.7, 240.2, 320.1; 514/2, 12; 530/415, 418, 419, 420, 427, 399.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CELL, VOLUME 66, NO. 2, ISSUED 1991, D. J. GLASS ET AL., "TRKB MEDIATES BDNF-NT-3-DEPENDENT SURVIVAL AND PROLIFERATION IN FIBROBLASTS LACKING THE LOW AFFINITY NGF RECEPTOR", PAGES 405-413, NO. 8620154, BIOSIS NO. 85154, SEE ENTIRE DOCUMENT.	1-38
Y	NATURE, VOLUME 354, ISSUED 1991, MCDONALD ET AL., "NEW PROTEIN FOLD REVEALED BY A 2.3-A RESOLUTION CRYSTAL STRUCTURE OF NERVE GROWTH FACTOR", PAGES 411-414, SEE THE ABSTRACT, NO. 9070399, BIOSIS NO. 93055399, SEE ENTIRE DOCUMENT.	1-38
Y	ISSUED JUNE 1991, REGENERON PHARMACEUTICALS INC., KAROLINSKA INSTITUTE, "MARKETS COMPOUNDS FOR TREATMENT OF NEURODEGENERATIVE DISEASES", PAGE 33. SEE THE ABSTRACT NO. 03257056, DIALOG FILE 16,	1-38

Further documents are listed in the continuation of Box C.

See patent family annex.

Special categories of cited documents:			
'A'	document defining the general state of the art which is not considered to be part of particular relevance	'T'	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
'E'	earlier document published on or after the international filing date	'X'	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
'L'	document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	'Y'	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
'O'	document referring to an oral disclosure, use, exhibition or other means	'&'	document member of the same patent family
'P'	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

16 APRIL 1993

Date of mailing of the international search report

05 MAY 1993

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A. CLASSIFICATION OF SUBJECT MATTER:

IPC (5):

A01N 37/18; A61K 37/00, 37/24, 37/36; A23J 1/00; C07K 3/00, 13/00, 15/00, 17/00; C12N 5/00, 15/00; C12P 21/06.

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/69.7, 240.2, 320.1; 514/2, 12; 530/415, 418, 419, 420, 427, 399.